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(71) Applicant *(for all designated States except US)*: FARMITALIA CARLO ERBA S.R.L. [IT/IT]; Via Carlo Imbonati, 24, I-20159 Milan (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : DE CASTIGLIONE, Roberto [IT/IT]; Via Domenichino, 38, I-20149 Milan (IT). GOZZINI, Luigia [IT/IT]; Viale Stelvio, 27/4, I-20159 Milan (IT). LUCIETTO, Pierluigi [IT/IT]; Via Buonarroti, 6, I-20145 Milan (IT). CORRADI, Fabio [IT/IT]; Via Marchiondi, 7, I-20122 Milan (IT). CIOMEI, Marina [IT/IT]; Via del Molo, 1, I-27020 Torre d'Isola (IT). MOLINARI, Isabella [IT/IT]; Via Elba, 28, I-20144 Milan (IT).

(74) Agents: KOLB, Helga et al.; Hoffmann, Eitle & Partner, Arabellastrasse 4, D-8000 Munich 81 (DE).

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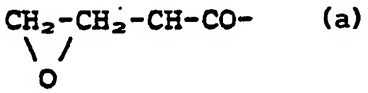

(a)

(57) Abstract

A peptide of formula (I), wherein R is a group of the formula 4-$(ClCH_2CH_2)_2N$-C_6H_4-CO-, 3-$(ClCH_2CH_2)_2N$-C_6H_4-CO-, $ClCH_2CH_2NHCO$-, $ClCH=CH$-CO-, $BrCH=CH$-CO-, $CH_2=CClCO$-, CH_2CBrCO- (cis/trans isomers), (a), $CH=C$-CO-, $ClCH_2CH_2CH_2n(NO)CO$-, or $ClCH_2CO$-$CH(R_1)NHCO(CH_2)_2CO$-; A = valence bond, or Gly, Leu-Gly, Arg-Leu-Gly, Gln-Arg-Leu-Gly; B = valence bond or Asn or Thr; C = Gln or His; X = Gly or ala; Y = valence bond or His(R_2), his(R_2), Phe, phe, Ser, ser, Ala or ala; T = valence bond or Leu, leu, Phe or phe; W is a group of the formula OH, NH_2, $NH(CH_2)_4CH_3$, $H(CH_2)_2C_6H_5$, Met-R_3, Leu-R_3, Ile-R_3, or Nle-R_3; R_1 = H, linear or branched alyphatic chain having for 1 to 11 carbon atoms, benzyl or phenyl group; R_2 = H or Tos, Dnp or Bzl and R_3 = NH_2, OH, OCH_3 or $NHNH_2$ and pharmaceutically acceptable salts are bombesin antagonists. Their preparation and pharmaceutical compositions containing them are also described.

* See back of page

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IRREVERSIBLE BOMBESIN ANTAGONISTS

The present invention relates to peptide derivatives, to pharmaceutical compositions containing them, to processes for their preparation, and to their application as therapeutic agents.

In this specification symbols and abbreviations are those commonly used in peptide chemistry (see Eur.J. Biochem. (1984) 138, 9-37). Consequently, the three-letter amino acid symbols denote the L configuration of chiral amino acids. D-amino acids are represented by small letters: e.g., ala = D-Ala. Other symbols and abbreviations used are: AA, amino acid; AcOEt, ethylacetate; BBS, bombesin; Boc, t-butoxycarbonyl; BuOH, n-butyl alcohol; BOP, benzotriazolyloxy-tris[dimethylamino]phosphonium hexafluorophosphate; DCC, N,N'-dicyclohexylcarbodiimide; DMF, dimethylformamide; DMSO, dimethylsulfoxide; Dnp, 2,4-dinitrophenyl; EGF, epidermal growth factor; EtOH, ethyl alcohol; FAB (or FD)-MS, fast atom bombardment (or field desorption) mass spectrometry; ECC, ethylchlorocarbonate; Et_2O, diethylether; Glp, L-pyroglutamic acid; h-GRP (or p-GRP), human (or porcine) gastrin releasing peptide; HOBt, 1-hydroxybenzotriazole; I.D., internal diameter; MeOH, methyl alcohol; m.p., melting point; n.d., not determined; Nle, L-norleucine; NMM, N-methylmorpholine; NMR, nuclear magnetic resonance; OSu,

N-hydroxysuccinimidyl; PE, petroleum ether 40°-70°; RP-HPLC, reversed phase high performance liquid chromatography; SCLC, small cell lung carcinoma; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography; Tos, p-toluensulphonyl.

The invention provides a peptide of the formula I:

R—A—B—C—Trp—Ala—Val—X—Y—T—W I

wherein

R represents a group of the formula

4-$(ClCH_2CH_2)_2N$-C_6H_4-CO-, 3-$(ClCH_2CH_2)_2N$-C_6H_4-CO-,
$ClCH_2CH_2NHCO$-, ClCH=CH-CO-, BrCH=CH-CO-, CH_2=CClCO-,
CH_2=CBrCO- (either cis or trans isomers),
CH_2-CH-CH_2-CO-, CH ≡ C-CO-, $ClCH_2CH_2N(NO)CO$-,
\ /
O or $ClCH_2CO$-$CH(R_1)NHCO(CH_2)_2CO$-;

A represents a valence bond, or a Gly, Leu-Gly, Arg-Leu-Gly, Gln-Arg-Leu-Gly residue,

B represents a valence bond or a Asn or Thr residue;

C represents a Gln or His residue,

X represents a Gly or ala residue;

Y represents a valence bond or a His(R_2), his(R_2), Phe, phe, Ser, ser, Ala or ala residue;

T represents a valence bond or a Leu, leu, Phe or phe residue;

W represents a group of the formula OH, NH_2, $NH(CH_2)_4CH_3$, $NH(CH_2)_2C_6H_5$, Met-R_3, Leu-R_3, Ile-R_3, or Nle-R_3,

R_1 represents hydrogen atom, linear or branched alyphatic chain having for 1 to 11 carbon atoms, benzyl or phenyl group,

R_2 represents hydrogen atom or Tos, Dnp or Bzl group and

R_3 represents an amino, hydroxy, methoxy or hydrazino group.

Salts of these peptides with pharmaceutically acceptable acids are within the scope of the invention. Such acid addition salts can be derived from a variety of inorganic and organic acids such as sulfuric, phosphoric, hydrochloric, hydrobromic, hydroiodic, nitric, sulfamic, citric, lactic, pyruvic, oxalic, maleic, succinic, tartaric, cinnamic, acetic, trifluoracetic, benzoic, salicylic, gluconic, ascorbic and related acids.

Preferred alyphatic chains which R_1 may represent include methyl, ethyl, n-propyl, iso-propyl, n-butyl and iso-butyl groups.

The synthesis of the peptides of the invention may be accomplished by classical solution methods. The synthesis consists essentially of appropriate successive condensations of protected amino acids or

peptides. The condensations are carried out so that the resulting peptides have the desired sequence of amino acid residues.

The amino acids and peptides, which can be condensed according to methods known in peptide chemistry, have the amino and carboxyl groups, not involved in peptide bond formation, blocked by suitable protecting groups capable of being removed by acid or alkali treatment or by hydrogenolysis.

For the protection of the amino group the following protective groups may, for example, be employed: benzyloxycarbonyl, t-butoxycarbonyl, trityl, formyl, trifluoracetyl, o-nitrophenylsulphenyl, 4-methyloxybenzyloxycarbonyl, 9-fluorenylmethoxycarbonyl, 3,5-dimethoxy-α-α'-dimethylbenzyloxycarbonyl or methylsulphonylethoxycarbonyl.

For the protection of the carboxyl group the following protective groups may, for example, be employed: methyl, ethyl, t-butyl, benzyl, p-nitrobenzyl or fluorenylmethyl, amide, hydrazide, t-butoxycarbonyl hydrazide or benzyloxycarbonyl hydrazide.

The hydroxy functions of hydroxy amino acids and the imino function of histidine may be protected by suitable protecting groups (throughout all the synthesis or only during a few steps) or may be unprotected. For the protection of the hydroxy function the following protective groups may, for example, be employed; t-butyl, benzyl, acetyl. For the protection of the imidazole imino function the following groups may, for example, be used: 2,4-dinitrophenyl, tosyl, benzyl. De-protecting reactions are carried out according to

methods known per se in peptide chemistry.

The condensation between an amino group of one molecule and a carboxyl group of another molecule to form the peptidic linkage may be carried out through an activated acyl-derivative such as a mixed anhydride, an azide or an activated ester, or by direct condensation between a free amino group and a free carboxyl group, in the presence of a condensing agent such as dicyclohexylcarbodiimide, alone or together with a racemization preventing agent, such as N-hydroxysuccinimide or 1-hydroxybenzotriazole, or together with an activating agent such as 4-dimethylamino-pyridine. The condensation may be carried out in a solvent such as dimethylformamide, dimethylacetamide, pyridine, acetonitrile, tetrahydrofuran or N-methyl-2-pyrrolidone.

The reaction temperature may be from -30°C to room temperature. The reaction time is generally from 1 to 120 hours.

The scheme of synthesis, the protecting groups and condensing agents are selected so as to avoid the risk of racemization.

Biological activity

The peptides of the present invention are endowed with potent antagonism versus "in vitro" and "in vivo" effects induced by bombesin, such as contraction of smooth musculature, modification of behaviour of central origin and mitogenesis.

Bombesin (BBS) is a tetradecapeptide of formula Glp-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2, originally isolated from the skin of a frog. The biological activity resides in the

C-terminal part of the molecule: BBS(6-14)nonapeptide is as active as the parent compound. The human counterpart of bombesin is a 27 amino acid peptide, known as gastrin-releasing peptide (h-GRP). Bombesin and bombesin-like peptides display a number of biological activities (J.H. Walsh (1983) in "Brain Peptides", D.T. Krieger, M.J. Brownstein and J.B. Martin (eds), Wiley Interscience Publ., pp. 941-960), including autocrine growth-promoting effects on human small cell lung carcinoma (SCLC) (F. Cuttitta et al. (1985) Cancer Survey, 4, 707-727), autocrine and/or paracrine stimulation of human prostatic cancer cell proliferation (M. Bologna et al., Cancer, in press) and modulation of the EGF receptor (I. Zachary and E. Rozengurt (1985) Cancer Surveys, 4, 729-765).

A bombesin antagonist, by competing with the natural ligand for the receptor(s), would inhibit the triggering of the cascade of events leading to abnormal cell proliferation.

Different approaches in this direction have been followed by different research groups. A series of C-terminal bombesin nona- and decapeptides, characterized by amino acid deletion, inversion or substitution, has been the object of a previous patent application by our side (EP Patent Application n° 89102283.2). These peptides, however, like other BBS antagonists, usually show moderate affinity for the BBS receptor.

The compounds of the present invention, having an alkylating moiety behave as receptor antagonists either when given in combination with bombesin or when administered 24 hours before bombesin challenge.

Biological test results

The binding affinity of the compounds of the present invention for the bombesin receptors was determined on mouse Swiss 3T3 fibroblasts (I. Zachary and E. Rozengurt (1985) Proc. Natl. Acad. Sci. USA, 82, 7616-7620) (Table 1).

The effect on mitogenesis was determined in quiescent and confluent Swiss 3T3 cells maintained in serum free medium (A.N.Corps et al (1985) Biochem J. 231, 781-785). In a first set of experiments, analogues are given alone or in combination with bombesin. In a second set of experiments, cells are pre-treated with the alkylating peptides, washed, left at 37°C for 24 hours and then challenged with bombesin. In both cases, DNA synthesis was evaluated as [H^3]thymidine incorporation (Table 2).

Mitogenic effect of bombesin and its analogues were also evaluated as activation of the protein-tyrosin kinase that phosphorylates a 115 KD protein (p115) associated with the bombesin receptor complex (D. Cirillo et al. (1986) Mol.Cell. Biol. 6, 4641- 4649) (Table 3).

In addition, exposure to these peptides in the 0.1-50 µM range was associated with significant reduction in the growth of SCLC cell lines (such as NCI-H345, NCI-N592, NCI-H128), as well as of prostatic carcinoma cell lines (such as DU145 and PC3).

Parenteral administration of these peptides at doses ranging between 1 ng/kg - 100 mg/kg to nude mice was associated with significant growth reduction of the above mentioned transplanted human SCLC and prostatic carcinoma cell lines.

TABLE 1

BINDING AFFINITY OF BOMBESIN ALKYLATING ANALOGUES ON MOUSE SWISS 3T3 FIBROBLASTS

COMPOUND	ID_{50} (nM)
I	1363 ± 266
II	438 ± 111
III	1815 ± 330
IV	0.7 ± 0.2
V	1336 ± 199
VI	648 ± 290

Reference peptides:

BBS	12.6 ± 3.8
Spantide	11100
[pro^2]Spantide	14000
[Leu$^{13}\psi$(CH_2-NH)Leu^{14}]BBS	214 ± 30

TABLE 2

[H³]THYMIDINE INCORPORATION IN MOUSE SWISS 3T3 FIBROBLASTS

COMPOUND	FOLD INCREASE OVER BASAL VALUE				% INHIBITION IN THE PRESENCE OF 25nM BBS			
					A		B	
	5nM	50nM	0.5μM	5 μM	0.5μM	5 μM	0.5μM	5 μM
I	N.D.	N.D.	0.6	0.6	9 ± 6	12±3	0	
II	N.D.	N.D.	1.0	1.0	0	50±10	0	
III	N.D.	N.D.	2.3	3.4	0	0	51±11	53+8
IV	N.D.	N.D.	3.2	2.2	13±8	32±9	0	20±12
V	N.D.	1.8.	1.8	2.5	0	0	0	0
VI	N.D.	1.0	1.0	1.0	0	0	39±14	29±10
Reference peptides:								
BBS	3.0±1							
[Leu$^{13}\psi$(CH_2-NH)Leu^{14}]BBS			1	1	29±10	56±4	0	0

A= analogues are given in combination with BBS

B= cells are pre-treated with analogues, washed, left at 37°C for 24 h and then challenged with BBS

TABLE 3

PHOSPHORYLATION OF THE p115 PROTEIN ASSOCIATED WITH THE BOMBESIN RECEPTOR

COMPOUND	MINIMAL ACTIVE DOSE (nM)
I	> 4000
II	> 1000
III	500
IV	500
V	> 2000
VI	> 1000

Reference peptides:

BBS	3
Spantide	> 10000
[pro^2]Spantide	> 10000
[Leu$^{13}\psi$(CH_2-NH)Leu^{14}]BBS	> 200

The peptides of the formula I, therefore, find application in the therapy of human neoplasms which are modulated in their growth and progression by peptides of the GRP family, either directly or in concert with other growth factors.

In addition, these alkylating analogues can be used in the management of the clinical symptoms associates with these deseases and due to hypersecretion of GRP-like peptides.

The compounds of the invention can be administered by the usual routes, for example, parenterally, e.g. by intravenous injection or infusion, or by intramuscular, subcutaneous, intracavity and intranasal administration.

The dosage depends on the age, weight and condition of the patient and on the administration route.

On the basis of the "in vitro" and "in vivo" data in mice it can be estimated that the therapeutic doses in humans will be in the range 1 ng/kg - 100 mg/kg, once to 6 times daily.

The invention also provides pharmaceutical compositions containing a compound of formula (I) as the active substance, in association with one or more pharmaceutically acceptable excipients.

The pharmaceutical compositions of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For instance, solutions for intravenous injection or infusion may contain as carrier, for example, sterile water or, preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound, a pharmaceutically acceptable carrier, e.g., sterile water, olive oil, ethyl oleate, glycols (e.g., propylene glycol) and, if desired, a suitable amount of lidocaine hydrochloride.

Furthermore, according to the invention, there is provided a method of treating neuroendocrine neoplasms (such as small cell lung carcinoma and prostatic carcinoma) or the clinical symptoms associated with these diseases in patients in need of it, comprising administering to the said patients a composition of the invention.

Chemistry

Methods:

a) TLC was performed on pre-coated plates of silica gel 60 F_{254} (Merck), layer thickness 0.25 mm, length 20 cm, with the following eluents:

System A: n-butanol/acetic acid/water = 600/150/150 by volume

System B: chloroform/methanol/NH_4OH 30% = 500/346 154 by volume

System C: chloroform/methanol = 90/10 by volume

System D: dichloromethane/diethylether = 90/10 by volume

System E: acetonitrile/water/formic acid= 80/20/10.

b) Analytical RP-HPLC was performed on a Hewlett Packard Mod. 1084 apparatus on a LiChrosorb Hibar RP-18 column (Merck) 250 x 4 mm I.D., particle diameter 5 µ. The following eluents were used:

A= KH_2PO_4 20 mM, pH 3.5/acetonitrile 9/1 by volume

B= KH_2PO_4 20 mM, pH 3.5/acetonitrile 3/7 by volume.

The elution is programmed with a linear gradient from 60% to 90% B over a period of 20 min (System A) or from 30 to 70% B over a period of 15 min (System B), and then isocratically for 15 min, with a flow rate of 1 ml/min.

The peptides are characterized by their retention time (RT).

c) Preparative RP-HPLC was performed using a Delta Prep 3000 apparatus (Waters) on a Deltapak column (Waters), 300 x 19 mm I.D., particle diameter, 10 µ. The following eluents were used:

A= 0.05% TFA in water

B= 0.05% TFA in acetonitrile/water 7/3 by volume.

Flow rate= 24 ml/min; detection wavelength= 220 nm.

Elution methods are reported in the single examples.

In each case, fractions were checked by analytical RP-HPLC and those showing a purity greater than 98% were pooled. After removal of acetonitrile, the solution was lyophilized.

d) Amino acid analysis was carried out on acid hydrolysates (either at 110°C for 22 h in 6 N HCl + 0.1% phenol or at 100°C

for 16 h in 3 N mercaptoethansulfonic acid, both under N_2). Only natural amino acid residues were determined. Due to partial decomposition in normal hydrolysis conditions, Trp was determined only in hydrolysates with mercaptoethansulfonic acid.

Example 1

Preparation of

4-$(ClCH_2CH_2)_2NC_6H_4CO$-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 (I).

25.2 mg (0.096 mmol) of [p-bis(2-chloroethyl)amine]benzoic acid were dissolved in 5 ml of distilled DMF and 60 mg (0.064 mmol) of H-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 . HCl (EP Patent Appl. N. 89102283.2) were successively added; the solution was cooled to 5°C and 0.0176 ml of NMM (0.16 mmol) and 0.0425 g of BOP (0.096 mmol) were added. The reaction mixture was stirred at room temperature overnight, then it was poured drepwise into 100 ml of a 10% solution of citric acid at 5°C. The mixture was stirred for 1 h at a temperature below 10°C, then filtered and washed with water to neutrality: 66.1 mg of crude product (90% yield) were obtained. The product was purified by preparative RP-HPLC, running a gradient from 60% to 90% of eluent B in eluent A over 30 min (flow rate, 35 ml/min): 33 mg of product I (50% yield) were obtained: Rf_A 0.61; RT_A= 8.66; FAB-MS: m/z 1147 (MH^+); AA ratios: Thr 0.98 (1), Glu 1, Ala 0.97 (1), Val 0.95 (1), Gly 1.04 (1), Leu 1.02 (1), Met 0.90 (1) (Trp n.d.).

Example 2

Preparation of
4-($ClC_2CH_2)_2NC_6H_4CO$-Thr-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2 (II).

0.2 g (0.216 mmol) of H-Thr-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2 . HCl (prepared following the methodology described in our EP Patent Appl. N. 89102283.2 for H-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 . HCl) were reacted with 0.085 g (0.324 mmol) of [p-bis(2-chloroethyl) amine]benzoic acid, g 0.143 (0.324 mmol) of BOP and 0.059 ml of NMM (0.537 mmol) in 17 ml of distilled DMF, as described in example 1. The crude product was purified by preparative RP-HPLC, running a gradient from 45% to 90% of eluent B in eluent A over 20 min: 0.122 g (50% yield) of product II were obtained: Rf_A 0.75; RT_A 10.39; FAB-MS: m/z 1129 (MH^+); AA ratios: Thr 0.97 (1), Glu 1, Ala 0.94 (1), Val 0.96 (1), Gly (1), Leu 0.92 (1), Nle 0.87 (1) (Trp n.d.).

Example 3

Preparation of
4-($ClCH_2CH_2)_2NC_6H_4CO$-Thr-Gln-Trp-Ala-Val-Gly-His(Dnp)-Leu-Met-NH_2 (III).

From 63 mg (0.24 mmol) of [p-bis(2-chloroethyl)amine]benzoic acid and 200 mg (0.16 mmol) of HCl . H-Thr-Gln-Trp-Ala-Val-Gly-His-(Dnp)-Leu-Met-NH_2 [prepared from Boc-Thr-Gln-Trp-Ala-Val-Gly-OH

(our EP Patent Application N. 89102283.2) and HCl . H-His(Dnp)-Leu-Met-NH_2 (F. Angelucci and R. de Castiglione (1975) Experientia, 507-508) following the procedure described in the same patent application for analogous compounds] and operating as described in Example 1, 161 mg (70%) yield) of crude compound III were obtained. The product was purified by preparative RP-HPLC running a gradient from 60% to 90% of eluent B in eluent A over 20 min: 60 mg (26% yield) of product III were obtained : Rf_A 0.70; RT_A 14.45; RT_B 24.82; FAB-MS : m/z 1451 (MH^+); AA ratios: Thr 1.05 (1), Glu 1, Gly 1.03 (1), Ala 0.99 (1), Val 0.95 (1), Met 0.90 (1), Leu 0.97 (1), (Trp and His n.d.)

Example 4

Preparation of

4-$(ClCH_2CH_2)_2NC_6H_4CO$-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (IV).

65 mg (0.05 mmol) of compound (III) were suspendend in 3 ml of 0.02 M phosphate buffer pH 8 and treated with of 2-mercapto-ethanol. The resulting clear solution was left to react for 20 min, then poured with stirring into 300 ml Et_2O. The precipitate was filtered, thoroughly washed with Et_2O, then distributed between Bu-OH and water. The organic phase was concentrated to small volume and the peptide precipitated by dilution with AcOEt/Et_2O: 50 mg of the crude compound (IV) (71% yield) were obtained. The product was purified by preparative RP-HPLC running a gradient from 40% to 90% of eluent B in

eluent A over 30 min: 25 mg (36% yield) of IV were obtained : Rf_A 0.39; RT_A 9.26 RT_A 9.26 ; RT_B 19.41; FAB-MS : m/z 1284 (MH^+); AA ratios: Thr 1.05 (1); Gln (1), Gly 1.03 (1), Ala 0.99 (1), Val 0.95 (1), Met 0.88 (1), His 0.91 (1) (Trp n.d.).

Example 5

Preparation of

$ClCH_2CH_2N(NO)CO$-Thr-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2 (V).

Step 1

$ClCH_2CH_2NHCO$-OSu (Va)

5.75 g (0.05 mol) of N-hydroxysuccinimide were dissolved in 125 ml of AcOEt and made to react at 0°C with 12.92 ml (0.075 mmol) of N-ethyl-diisopropylamine and 5.12 ml (0.060 mol) of 2-chloroethyl-isocianate, added dropwise consecutively. The reaction mixture was left 48 hours under vigorous stirring, then the solvent was evaporated and the residue distributed between AcOEt and water. The organic layer was dried over Na_2SO_4 and evaporated to small volume. The product crystallized upon standing in the cold: 7.68 g (70% yield) of compound (Va) were obtained: m.p. 104-107°C; Rf_C 0.43; FAB-MS: m/z 220 ($M^{+\cdot}$); ^{1}H-NMR (200 MHz, DMSO-d_6) δ(ppm): 8.57 (t, 1H, $CH_2\underline{NH}CO$), 3.64 (t, 2 H, $Cl\underline{CH_2}CH_2$), 3.39 (m, 2 H, $CH_2\underline{CH_2}NH$), 2.75 (s, 4 H, succinimide); elemental analysis ($C_7H_9N_2O_4Cl$): C 38.10 (38.11), H 4.10 (4.11), N 12.67 (12.70), Cl 16.00 (16.07).

$ClCH_2CH_2N(NO)CO$-OSu (Vb)

1.100 g (0.005 mol) of compound (Va) were dissolved in 12 ml of CH_2Cl_2 and made to react at 0°C with 0.800 ml (0.010 mol) of pyridine and 14.0 ml (0.020 mol) of 1.4 N NOCl in CH_2Cl_2. The reaction mixture was left under vigorous stirring for five fours, then was washed with cold water, dried over Na_2SO_4 and evaporated to small volume. The crystallization began at room temperature and was completed in the cold. 0.80 g (63%) of compound (V b) were obtained: m.p. 101-103°C; Rf_c 0.87; FAB-MS: m/z 249 ($M^{+\cdot}$); ^{1}H-NMR (200 MHz, DMSO-d_6)δ (ppm): 4.13 (t, 2 H, $ClCH_2CH_2$), 3.69 (t, 2 H, CH_2CH_2NNO), 2.89 (s, 4 H, succinimide); elemental analysis ($C_7H_8N_3O_5Cl$): C 33.70 (33.68), H 3.25 (3.23), N 16.82 (16.83), Cl 14.80 (14.20).

$ClCH_2CH_2N(NO)CO$-Thr-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2 (V)

0.185 g (0.2 mmol) of HCl . H-Thr-Gln-Trp-Ala-Val-Gly-Leu-Nle-NH_2 (see example 2) were suspended in a mixture of 0.5 ml of hexamethylphosphorylamide and 0.5 ml of N-methylpyrrolidone and made to react at room temperature with 0.028 ml (0.2 mmol) of triethylamine followed by 0.100 g (0.4 mmol) of compound (Vb). After a 2 h stirring, the reaction mixture was diluted with water: 0.150 g (73% yield) of crude compound (V) were obtained. A 50 mg sample was purified by preparative RP-HPLC running a gradient from 10% to 90% eluent A in eluent B over 30 min: Rf_B 0.59; RT_B 16.10; FAB-MS: m/z 1020 ($MH^{+\cdot}$); ^{1}H-NMR (200 MHz, DMSO-d_6)δ (ppm) i.a.: 3.60 (t, 2 H, $ClCH_2CH_2$); AA ratios: Thr 0.91 (1), Glu 1.07 (1), Ala

1.03 (1), Val 1, Gly 1.02 (1), Leu 1.01 (1), Nle 0.90 (1) (Trp n.d.).

Example 6

Preparation of
$CH_3(CH_2)_3CH(COCH_2Cl)NHCO(CH_2)_2CO$-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 (VI)

Step 1

Boc-Nle-$CH=N_2$ (VIa)

4.60 g (0.02 mol) of Boc-Nle-OH in 40 ml anhydrous THF were made to react at -12°C with 2.20 ml (0.02 mol) of NMM and 2.62 ml (0.02 mol) of isobutylchlorocarbonate. After 1 min the reaction was quenched by adding 50 ml Et_2O and cooling the solution to -35°C. Salts were filtered off and the filtrate cooled to -70°C.
In a separate vessel, 3.96 g (0.06 mol) of KOH dissolved in 20 ml of 50% EtOH were added dropwise in 10 min at 0°C to a solution of 6.43 g (0.03 mol) of N-methyl-N-nitroso-p-toluensulfonamide in 100 ml Et_2O and 10 ml ethylenglycol monomethylether. The so formed diazomethane was distilled directly into the reaction vessel containing the preformed and chilled mixed anhydride. After stirring the reaction mixture at 0°C for 20 hours, the solvent was evaporated under reduced pressure and the residue distributed between AcOEt and 0.5 M aqueous $KHCO_3$. The organic layer was washed

to neutrality with brine, dried over Na_2SO_4 and evaporated in vacuo to give an oil that crystallized upon standing in the cold: 2.9 g (57% yield) of compound (VIa) were obtained: m.p. 83-85°C; Rf_D 0.51; ^{1}H-NMR (200 MHz, DMSO-d_6)δ (ppm) i.a.: 6.00 (s, 1 H, CH=N_2); FD-MS: m/z 255 ($M^{+\cdot}$); elemental analysis ($C_{12}H_{21}N_3O_3$): C 56.50 (56.45), H 8.30 (8.29), N 16.47 (16.46).

step 2

HCl . H-Nle-CH_2Cl (VIb)

2.4 g (0.01 mol) of Boc-Nle-CH=N_2 (VIa) were dissolved in 20 ml of 4.4 N HCl in dioxane. After 5 min at room temperature the product was precipitated with Et_2O: 1.56 g (78% yield) of compound (VIb) were obtained: m.p. 153-154°C; Rf_E 0.63; ^{1}H-NMR (200 MHz, DMSO-d_6) δ (ppm) i.a.: 4.84, 4.74 (two d, 2 H, CH_2-Cl); FD-MS: m/z 199 ($M^{+\cdot}$); elemental analysis ($C_7H_{15}NOCl_2$); C 42.02 (42.01), H 7.54 (7.55), N 6.96 (6.99), Cl 35.42 (35.43).

HOOC-CH_2CH_2-CO-Nle-CH_2Cl (VIc)

0.60 g (3 mmol) of HCl . H-Nle-CH_2Cl (VIb) and 0.33 ml (3 mmol) of NMM were dissolved in 10 ml of DMF and made to react at 0°C with 0.30 g (3 mmol) of succinic anhydride in 5 ml of DMF added dropwise in 10 min. After 30 min the reaction mixture was allowed to reach room temperature and kept under stirring 4 h longer. The solvent was evaporated under reduced pressure, the residue taken up in AcOEt and, after filtration of the insoluble material, washed with

0.02 M HCl, brine, and finally dried over Na_2SO_4. Evaporation of the solvent and trituration with Et_2O/PE gave 0.35 g (44% yield) of compound (VIc): m.p. 114-116°C; Rf_B 0.33; ^{1}H-NMR (200 MHz, DMSO-d_6) δ (ppm) i.a.: 4.57 (s, 2H, CH_2-Cl); elemental analysis ($C_{11}H_{18}NO_4Cl$): C 49.74 (50.10), H 5.34 (5.31), Cl 13.16 (13.44).

$CH_3(CH_2)_3CH(COCH_2Cl)NHCO(CH_2)_2CO$-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 (VI)

100 mg (0.106 mmol) of HCl . H-Thr-Gln-Trp-Ala-Val-Gly-Leu-Met-NH_2 (EP Patent Appl. N. 89102283.2) and 0.006 ml (0.106 mmol) NMM in 2 ml DMF were made to react at 0°C with 31 mg (0.120 mmol) HOOC-$(CH_2)_2$CO-Nle-CH_2Cl (VIc), 19 mg (0.143 mmol) HOBT and 27 mg (0.133 mmol) DCC. The reaction mixture was then stirred for 4 days at room temperature. The solvent was evaporated under reduced pressure, the residue triturated with warm isopropyl alcohol, and the insoluble material isolated by filtration: 50 mg (40% yield) of compound (VI) were obtained: Rf_A 0.42; RT_B 15.10; FAB-MS: m/z 1149 (MH^+); AA ratios: Thr 0.95 (1), Glu 1, Ala 1.08 (1), Val 0.97 (1), Gly 1.01 (1), Leu 1.00 (1), Met 0.93 (1) (Trp n.d.).

CLAIMS

1. A peptide of the formula I:

R—A—B—C—Trp—Ala—Val—X—Y—T—W I

wherein R represents a group of the formula 4-$(ClCH_2CH_2)_2N$-C_6H_4-CO-, 3-$(ClCH_2CH_2)_2N$-C_6H_4-CO-, $ClCH_2CH_2NHCO$-, ClCH=CH-CO-, BrCH=CH-CO-, CH_2=CClCO-, CH_2=CBrCO- (either cis or trans isomers),

CH_2-CH-CH_2-CO-, HC ≡ C-CO-, $ClCH_2CH_2CH_2N(NO)CO$-,
\ /
O or $ClCH_2CO$-$CH(R_1)NHCO(CH_2)_2CO$-;

A represents a valence bond, or a Gly, Leu-Gly, Arg-Leu-Gly, Gln-Arg-Leu-Gly residue;

B represents a valence bond or a Asn or Thr residue;

C represents a Gln or His residue;

X represents a Gly or ala residue;

Y represents a valence bond or a His(R_2), his(R_2), Phe, phe, Ser, ser, Ala or ala residue;

T represents a valence bond or a Leu, leu, Phe or phe residue;

W represents a group of the formula OH, NH_2, $NH(CH_2)_4CH_3$, $NH(CH_2)_2C_6H_5$, Met-R_3, Leu-R_3, Ile-R_3, or Nle-R_3;

R_1 represents hydrogen atom, linear or branched alyphatic chain having for 1 to 11 carbon atoms, benzyl or phenyl group;

R_2 represents hydrogen atom or Tos, Dnp or Bzl group and

R_3 represents an amino, hydroxy, methoxy or hydrazino group, and the pharmaceutically acceptable salts thereof.

2. A pharmaceutical composition comprising a peptide according to claim 1 or a pharmaceutically acceptable salt of such a peptide in admixture with a pharmaceutically acceptable diluent or carrier.

3. A process for the preparation of a peptide according to claim 1, the process comprising condensing amino acids and/or amino acid derivatives in the desired sequence and/or peptide fragments containing these amino acids or their derivatives in the desired sequence to give the desired peptide, the end carboxylic acid group being activated for the peptide linkage and the remaining groups being protected and deprotecting the resultant compound and/or converting the resultant peptide into a pharmaceutically acceptable salt thereof.

4. The use of the peptides according to claim 1 for the preparation of a pharmaceutical suitable in the therapy of human neoplasms.

5. The use of the peptides according to claim 1 for the preparation of a pharmaceutical useful as an antagonist versus effects induced by bombesin.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/00922

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 K 7/06, A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4331661 (W.E. MARKI et al.) 25 May 1982 see abstract, especially R1 --	1-5
Y	US, A, 4423236 (WELEBIR) 27 December 1983 see column 1, line 55 - column 2, line 15 --	1-5
Y	Cancer Research, vol. 35, March 1975, (Baltimore, US,), D.J. Reed et al.: "2-chloroethanol formation as evidence for a 2-chloroethyl alkylating intermediate during chemical degradation of 1-(2-chloroethyl-3-cyclohexyl-1-nitrosourea and 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea", ./.	1-5

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24th September 1990	17. 10. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	H. Ballesteros

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	pages 568-576, see page 568: "Introduction"; pages 573-575: "Discussion" --	
A	British Journal of Pharmacology, vol. 55, October 1975, (Basingstoke, Hants, GB), M. Broccardo et al.: "Relative potency of bombesin-like peptides", pages 221-227, see page 222, table 1 --	1-5
O,P,X	Peptides - Chemistry, Structture and Biology, Proceedings of the Eleventh American Peptide Symposium, 9-14 July 1989, La Jolla, California, edited by J.E. Rivier et al., ESCOM, (Leiden, NL), R. de Castiglione et al.: "Irreversible ligands for bombesin receptors", pages 168-170, see the whole article ----------	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9000922 SA 37760

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/10/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4331661	25-05-82	None	
US-A- 4423236	27-12-83	AU-A- 7532381	17-03-82
		AU-A- 7532481	17-03-82
		EP-A- 0057699	18-08-82
		EP-A- 0057700	18-08-82
		WO-A- 8200644	04-03-82
		WO-A- 8200642	04-03-82